

Detection of Insulin in Frozen Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[0.3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: M.O.M. Immunodetection Peroxidase Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-2200

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Mouse Monoclonal Anti-Insulin Antibody

Sigma-Aldrich

St. Louis, MO

www.sigmaaldrich.com

1-800-325-3010

Catalog # I2018

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum

BD Biosciences

San Jose, CA 95131

www.bdbiosciences.com

1-855-236-2772

Catalog # 557273

Staining Procedure

Positive Control Tissue: Pancreas (islets of Langerhans)

Stain Localization: Cytoplasmic

1. Cut each frozen section at 6µm and mount on a positively charged slide.
Immediately fix the section in Rapid Fixx solution for 7 seconds.
Rinse the slide thoroughly in tap water to remove excess fixative, and then place it in 1X wash buffer.
Once all the slides have undergone this process, proceed to step 2.
2. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
3. Quench endogenous peroxidase by placing the slides in 0.3% hydrogen peroxide for 30 minutes.
4. Rinse in 2 changes of 1X wash buffer for 5 minutes each.

M.O.M Peroxidase Kit

Exp. Date_____ New Kit: yes / no

5. Apply the blocking reagent from the M.O.M. Kit. Incubate for 1 hour at room temperature.
(Add 2 drops of the Mouse IgG Blocking Reagent to 2.5 ml of 1X PBS.)

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Avidin / Biotin Blocking Kit

Lot #_____ Exp. Date_____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X wash buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

M.O.M. Diluent: Add 600ul of the Protein Concentrate stock solution to 7.5 ml of 1X PBS. Use this as the diluent for the primary, negative, and secondary antibodies.

7. Apply the primary antibody at a 1:16,000 dilution. Incubate for 15 minutes at room temperature.
Lot #_____ Exp. Date_____

For negative control slides, dilute mouse IgG1 control serum so that it's IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:16,000 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 15 minutes at room temperature.

Lot #_____ Exp. Date _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
9. Apply the secondary antibody from the M.O.M. Kit. Incubate for 10 minutes at room temperature.
(Add 10ul of the Biotinylated anti-Mouse IgG Reagent to 2.5 ml of the M.O.M. Diluent).
10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

11. Apply the label complex from the M.O.M. Kit. Incubate for 5 minutes at room temperature.
(Add 2 drops of Reagent A to 2.5 ml of 1X PBS. Mix. Then add 2 drops of Reagent B and mix.
Prepare at least 30 minutes prior to use.)
12. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
13. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp. Date_____ New Kit: yes / no
14. Rinse the slides in tap water 3 minutes.
15. Counterstain with hematoxylin for 20 seconds.
16. Rinse the slides in tap water until water is clear.
17. Gently agitate slides in 1X wash buffer until the tissues turn blue.
18. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip

Updated 08/23/07